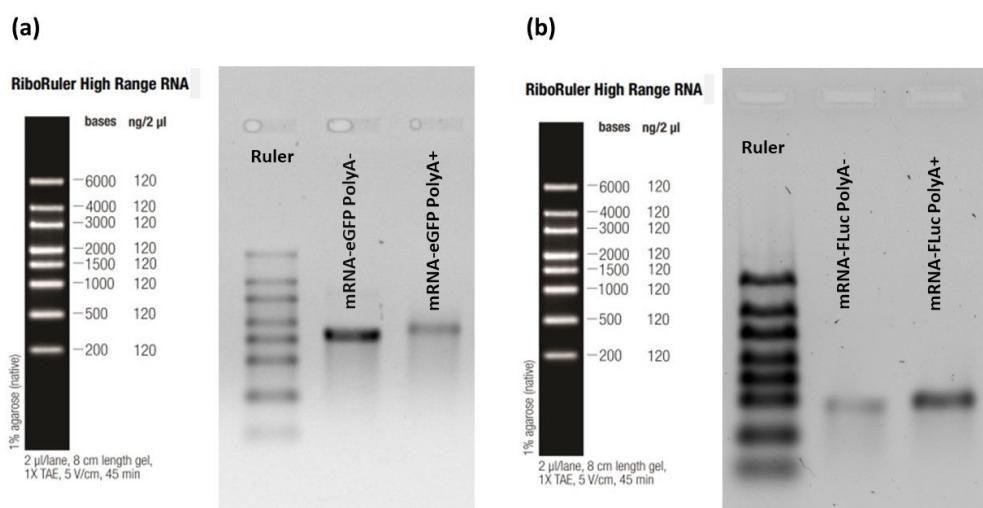


## Supplementary materials

### S1. Characterization of 2X3 by NMR

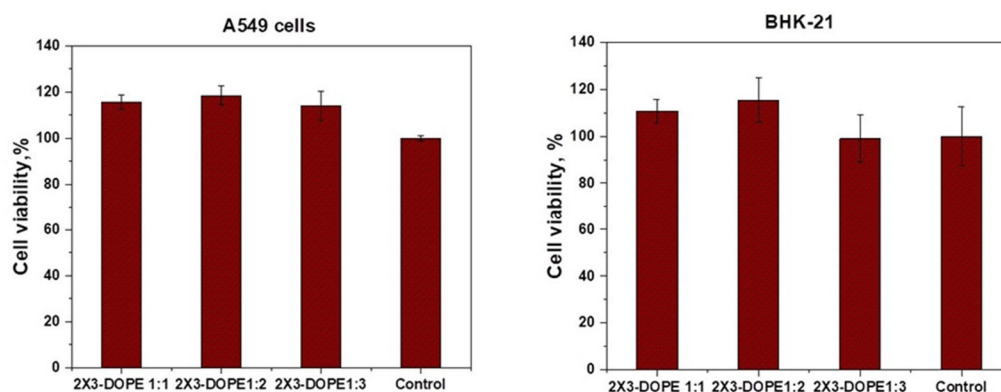
2X3: beige solid, mass-spectra: found  $m/z$  1226.3  $[M - 4 \text{ HCl} + \text{H}]^+$ , calculated for  $\text{C}_{78}\text{H}_{144}\text{Cl}_4\text{N}_6\text{O}_4$ : 1225.13  $[M - 4 \text{ HCl}]^+$ . NMR  $^1\text{H}$  (DMSO- $d_6$  -  $\text{CDCl}_3$ , 1 : 3 vol.): 0.61 (s, 6 H, 2 C(13)Me), 0.79 (d, 6 H,  $J = 6.5$ , 2 C(25)Me), 0.80 (d, 6 H,  $J = 6.5$ , 2 C(25)Me), 0.83 (d, 6 H,  $J = 6.5$ , 2 C(20)Me), 0.94 (s, 6 H, 2 C(10)Me), 0.91-2.04 (m, 76 H, protons Chol,  $\text{NHCH}_2(\text{CH}_2)_2\text{CH}_2\text{NH}$ , 2  $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$ , 2  $\text{CH}_2(\text{CH}_2)_4\text{CH}_2$ ), 2.12-2.35 (m, 4 H, 2  $\text{H}_2\text{C}(4)$  Chol), 2.40-2.61 (m, 8 H, 2  $\text{CH}_2\text{NHCH}_2$ ), 2.99-3.27 (m, 12 H, 2  $\text{CH}_2\text{NH}$ , 4  $\text{NCH}_2$ ), 4.34-4.49 (m, 2 H, 2 H(3) Chol), 4.57-4.70 (m, 2 H, 2 NH), 5.26-5.33 (m, 2 H, 2 H(6) Chol).

### S2. Quality assessment of mRNAs after in vitro transcription



**Figure S1.** Quality assessment of mRNAs after in vitro transcription by using 1% (w/v) agarose gel in 1× TBE buffer under denaturing conditions. (a) mRNAs encoding eGFP (mRNA-eGFP): Thermo Scientific RiboRulerHigh Range RNALadder #SM1821, mRNA-eGFP PolyA-, mRNA-eGFP PolyA+. (b) mRNAs encoding firefly luciferase (mRNA-FLuc): Thermo Scientific RiboRulerHigh Range RNALadder #SM1821, mRNA-FLuc PolyA-, mRNA-FLuc PolyA+.

### S3. Cytotoxicity studies



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**Figure S2.** Cell viability of A549 and BHK-21 cells at 24 hours after the treatment with the cationic liposomes. Error bars represent  $\pm$  SD (n=3).